

STUDIES ON GORAN TANNIN: PART III

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The chromatographically mobile constituents of the fresh goran bark have been studied. The constituents due to the overlapping rate of mobility in the paper chromatogram cannot be separated by the preparative technique. The constituents after spraying with the diagnostic reagents showed mainly the presence of phloroglucinol and catechol nuclei in the molecule and they yielded mainly phloroglucinol and protocatechuic acid as fusion products after alkali fusion. They yielded only cyanidin chloride on treatment with hydrochloric acid.

In the earlier communications,^{1,2} the chemical nature of the chromatographically immobile constituents (highly polymeric fractions) and some of the mobile constituents (low polymeric and monomeric fractions) of old goran bark has been reported. Alkali fusion of the immobile constituents yielded mainly phloroglucinol and protocatechuic acid. The immobile constituents were found to be mainly condensation polymers based upon 5, 7, 3', 4', tetrahydroxyflavan-3, 4-diol unit. One of the building units of some of the isolated mobile constituents of old goran bark has been found to be of leucocyanidin type.²

The present investigation was designed to see if there is any difference between the chromatographically mobile constituents of fresh and of old goran bark.

Previous work reported³ that when goran bark is extracted with methanol or any other organic solvent, most of the tannins remaining after the removal of

the solvent were insoluble in water. So, in the present experiment, water was used to make the extract.

Experimental*Preparation of the extract*

Finely crushed fresh goran bark (*Serriops roxburghiana*) (2 kg.) was soaked in water (8 l.) and allowed to stand for 48 hours at room temperature and the tan infusion was filtered through cotton wool. The bark residue was extracted in the same manner once more. The two consecutive extractions were then mixed and taken to complete dryness under reduced pressure. The above extract (100 g.) was purified by mixed solvent purification technique.⁴

Separation of the chromatographically mobile and immobile constituents

Purified tannin (50 g.) was dissolved with dry methanol (100 ml.). Excess ethyl ether (400 ml.) was then added to

it with stirring and the precipitate obtained was allowed to settle. The supernatant was then decanted and the precipitate was dissolved in a little methanol and precipitated as before. The process was repeated once more. The supernatants were then mixed and the excess solvent removed *in vacuo* to give a residue (4.8 g.) containing all the mobile constituents.

Paper chromatography of the mobile constituents:

A two dimensional chromatogram of the residue was then made by using Whatman No. 3 (18 × 22") by descending technique. *n*-Butanol: acetic acid: water (60:15:25) was used for the first dimension and the irrigation was continued (18 hours) till the solvent reached opposite edge of the paper and 2% acetic acid was used for the second dimension. The paper was dried and sprayed with diazotised *p*-nitroaniline. Ten spots were observed (Fig. 1) as compared to seven spots in the case of the tannins extracted from old goran bark. The spots were so close to each other that it was not possi-

ble to isolate even a single constituent in the pure state by the preparative technique followed previously.² Only constituents 3 and 4 of fresh bark corresponded to constituents D and F respectively of old bark.² Constituents A, B, C, E and G of old bark were found missing and new constituents viz., 1, 2, 5, 6, 7, 8, 9 and 10 were found to be present in fresh bark. In other words, most of the constituents of fresh bark were not observed at the same R_f as in the case of old bark. Different solvent systems were tried to effect better resolution of the constituents but without success.

Identification of constituents by spray reagents and other methods

Attempts were made to identify the basic nature of the different constituents using different spray reagents, fusion experiments and by conversion to anthocyanidin chlorides. For this, ten chromatograms of the mobile constituents were made in Whatman No. 3 (9 × 12") paper by running the papers with 6% acetic acid and *sec.* butanol: acetic acid: water (14:1:5) for the first and the

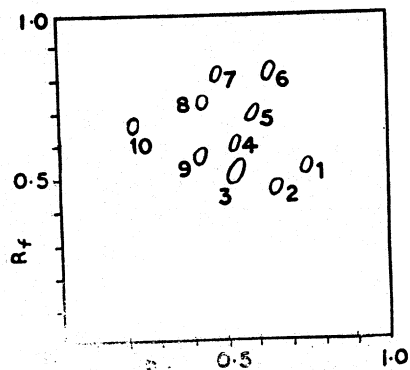


FIG. 1

1. *n*-butanol: acetic acid: water (60:15:25)
2. 2% acetic acid

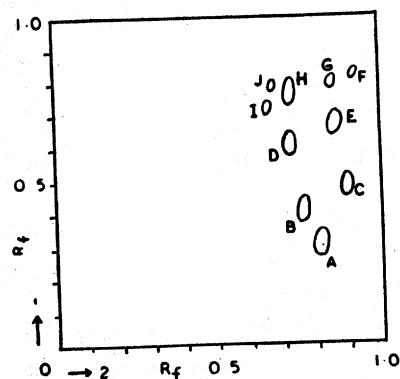


FIG. 2

1. 6% acetic acid
2. *sec.* butanol: acetic acid: water (14:1:5)

second dimensions respectively. The papers were then allowed to dry for 24 hours. One of the papers was then sprayed with diazotised *p*-nitroaniline.⁵ The ten spots were marked differently as A, B, C, D, E, F, G, H, I and J (Fig. 2). The papers were then sprayed with different spray reagents as follows, as suggested by Roux and Maih.⁶

(i) *Ferric ammonium sulphate (iron alum)*: A fresh solution of 3% salt in water was used. A, B, C, D, E and H showed green colour indicating catechol group in the constituents; others did not show any colour.

(ii) *Ammoniacal silver nitrate*:⁷⁻⁹ A, B, C, D, E and H yielded dark grey colour indicating catechol group in the constituents.

(iii) *Bis-diazotised benzidine*:¹⁰ All the spots were visible after spraying with this reagent. A, B, C, D, E and H showed scarlet maroon colours, which are indicative of catechin, gallo catechin, leucocyanidin and leucodelphinidin. But since green colour was developed by most of the compounds after spraying with iron alum and dark grey colour after spraying with ammoniacal silver nitrate, the spots presumably indicate catechol group only. Thus the above compounds may be catechin or leucocyanidin or their polymer. F and G (very weak spots) showed light yellow colours, which presumably are indicative of leucorobinetinidin or leucofisetinidin.

(iv) *Vanillin-toluene-p-sulphonic acid*:¹¹ A, B, C and H showed violet red colour even without heating; D and E showed violet red colour after heating for 3 minutes at 80-100°C. The immediate

violet red colours are indicative of substances like catechin, gallo catechin, leucocyanidin and leucodelphinidin having phloroglucinol nuclei in the constituents. But as there are no pyrogallol groups in the molecules, the constituents might be only catechin or leucocyanidin or their polymers.

From the above, it is clear that A-ring of most of the constituents (flavonoid compounds) is of phloroglucinol type and B-ring is of catechol type.

Semi-micro degradation of the constituents

The constituents were then subjected to semi-micro fusion so as to show the major compounds present in the fusion product.

5 g. potassium hydroxide was taken in a nickel basin and heated over a flame until it melted. The basin with the contents was then taken out of the flame and the tannin constituents (0.5 g.) were poured slowly in the molten alkali with stirring which was continued for another two minutes. The basin with the content was again held much above the flame for another 5 minutes so as to keep the alkali in the molten condition, stirring being continued all through. The fused constituents were then cooled in a strong current of air and dissolved in a little quantity of water (30 ml.). Sufficient 5N HCl was then added to make it acidic. The soluble degradation products were then separated into phenols and phenolic carboxylic acids as described by Roux.¹² These products were then resolved by descending chromatography on Whatman No. 3 using *n*-butanol : acetic acid : water (6:1:2) as solvent system.

Diazotised *p*-nitroaniline was used as spray reagent for the phenolic fraction and 2% ferric chloride solution for the phenolic acid fraction. Phloroglucinol (R_f 0.65, reddish brown), catechol (R_f 0.82, greenish brown) and protocathechuic acid (R_f 0.74, green) were identified in the chromatograms. The presence of above compounds was confirmed by other spray reagents like bis-diazotised benzidine and iron alum.

Micro degradation studies clearly indicate that most of the constituents have got phloroglucinol type of structure in the A-ring and catechol type of structure in B-ring.

Leucoanthocyanidin reaction of the constituents

The constituents were then studied for their leucoanthocyanidin following the method of Swain and Hillis.¹³ The constituents showed two absorption maxima, one at 450 $m\mu$ which was very weak and the other at 550 $m\mu$ which was very sharp, the former was identical with that of catechin and the latter with that of cyanidin.

The constituents after treatment with the leucoanthocyanidin reagent were streaked repeatedly on two narrow strips ($4 \times 18''$) of Whatman No. 3 chromatographic paper. One of the papers was developed with 90% formic acid: 3N HCl (1:1)¹⁴ and the other was similarly treated with Forestal solvent, viz., water: acetic acid: HCl (10:30:3). Two red spots were observed, one at R_f 0.24 and the other at R_f 0.64 when developed in the first solvent system. In the second system, the red spots corresponded to R_f 0.5 and 0.88. The red spot at R_f 0.24 in the first solvent system and

that in the second at R_f 0.5 corresponded with the one obtained for cyanidin. As also observed by Roux and Bill,¹⁵ and Ghosh and Barat² the other spot could not be identified with any known anthocyanidin.

Tanning potency of the constituents

The constituents were found to be highly soluble in water; a little quantity (0.2 g.) of the constituents was dissolved in water (50 ml.). A small piece of delimed goat skin was put in the liquor for 24 hours, when it was found to be completely penetrated. The piece was kept in the same liquor for two more days. The T_s of both treated and untreated delimed goat skin was then determined. The piece tanned with the constituents yielded 88°C as compared to 63°C for the control. The higher shrinkage temperature of the piece showed that most of the constituents are tannins.

Discussion and results

As all the mobile constituents of the fresh goran bark were found to be almost overlapping in the chromatogram, it was not possible to separate even a single constituent in pure state in mg. scale. The only difference between the previous work² and the present work is that the mobile constituents were derived in the previous case from the methanol extract of old bark which had low values and were easily isolated by preparative technique, were not found among the mobile constituents of fresh bark. Most of the constituents were found to give higher R_f values than those of old bark. Presumably, c

tients having smaller particle size are converted into bigger particles (polymeric product) due to the oxidation of the constituents and this might be the reason for more of low R_f and less number of constituents in the old bark.

From the chromatographic spots, it appeared that out of these ten constituents, six (A, B, C, D, E and H) constitute the major portion. The remaining four were found to be present only in traces. The six major constituents showed the presence of phloroglucinol and catechol when sprayed with diagnostic spray reagents. The other four constituents did not show the presence of any of the above compounds. It seems probable that these minor four constituents are either different compounds or are present in such traces that these cannot react with the spray reagent to give specific colouration.

The fusion products of the constituents showed the presence of only phloroglucinol and catechol in the phenolic fraction and protocathechuic acid in the phenolic acid fraction. Similarly, the leucoanthocyanidin reaction of the constituents showed the presence of mainly cyanidin and traces of catechin and catechin-like substances. No other anthocyanidin was detected.

From the above studies, it follows that there might be a few other flavonoid compounds amongst the mobile constituents but most of the constituents are presumably leucocyanidin polymerised to different degrees as was concluded in the case of the mobile constituents of old bark.

The higher shrinkage temperature of the piece tanned with the constituents

showed that most of the mobile constituents are tannins.

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